SECTION 1. Patrol Studies

Physiological stresses related to hypercapnia during patrols on submarines

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Schaefer, K. E. 1979. Physiological stresses related to hypercapnia during patrols on submarines. Undersea Biomed. Res. Sub. Suppl.: S15–S47.—Physiological studies on hypercapnic effects carried out on 13 Polaris patrols are summarized. The average CO_2 concentrations ranged from 0.7–1% CO_2; CO_2 was identified as the only environmental contaminant of the submarine atmosphere that has a direct effect on respiration in the concentration range found in the submarine atmosphere. A comparison has been made of physiological effects produced during 42 days of exposure to 1.5% CO_2 during laboratory studies (L.S.) with those observed during 50 to 60 days of exposure to 0.7–1% CO_2 on patrols (P.S.). A close similarity was found in the effects on respiration and blood electrolytes under both conditions. Respiratory minute volume was elevated by 40–63% because of increased tidal volume. The physiological dead space increased 60%. Vital capacity showed a trend toward a decrease. Studies of acid-base balance carried out during patrols demonstrated cyclic changes in blood pH and bicarbonate; pH and blood bicarbonate fell during the first 17 days of exposure, rose during the subsequent 20 days, and decreased again after 40 days. These cycles cannot be explained on the basis of known renal regulations in CO_2-induced acidosis and were not found during exposure to 1.5% CO_2. The hypothesis is advanced that these changes in acid-base balance are caused by cycles in CO_2 uptake and release in bones. The time constants of the bone CO_2 stores fit the observed length of cycles in acid-base balance. Correlation with cycles of calcium metabolism provides further support for this hypothesis. Red cell electrolytes showed similar changes under 1.5% CO_2 (L.S.) and 0.7–1% CO_2 (P.S.). Red cell sodium increased and potassium decreased. Moreover, red cell calcium also increased under both conditions. The significance of these red cell electrolyte changes in regard to changes in permeability and active transport remains to be clarified. An increased gastric acidity was found during patrol (exposure to 0.8–0.95% CO_2). The changes observed during patrols disappeared during the recovery periods.

respiration
acid-base balance
CO_2 response
blood electrolytes

During the first 10 years after the advent of the nuclear-powered submarine, from 1953 to 1963, great strides were made in the improvement of submarine habitability and atmosphere

control. Contaminants in the submarine atmosphere were determined and threshold limit values were developed for a 90-day period. Experience showed that toxicological hazards were prevented by the existing atmosphere control system, and there were no significant, acute problems in health or disease related to prolonged confinement in the sealed environment of the nuclear-powered submarines (Ebersole 1960; Kinsey 1960; Schulte 1961, 1963; Schaefer 1964).

During the second decade, from 1963 to 1973, submarine medical officers wrote nearly 900 patrol reports, which contain a great deal of experience in preventive medicine (Wilken 1969; Tansey, Wilson, and Schaefer 1979). Moreover, medical officers carried out a large number of physiological studies during patrols.

There are two reasons to summarize these medical experiences and physiological studies at this time: (1) The program requiring naval medical officers to be aboard each patrol will not be continued, and any new programs to be developed will benefit from the summary of previous experiences. (2) The medical experiences and results of physiological studies gathered during the submarine patrols represent a genuine contribution to preventive medicine as it concerns the general public. They provide base-line data, not otherwise available, on healthy subjects exposed to a technological environment for prolonged periods of time.

Characteristics of patrol studies

Included in this report are the results of 13 patrol studies carried out by several naval medical corps officers.* The patrol studies had of necessity a limited scope to succeed at all. In some cases only one function was studied at one point of the patrol (20 days) and this was then compared with control conditions and a point during the recovery period. Such data do not lend themselves to publication but provide valuable information within the framework of the program on submarine studies.

The patrol studies were difficult to do because the control data had to be taken during the hectic time prior to going to sea, and the recovery data had to be obtained immediately after the patrol, when everybody wanted to go on leave. Moreover, during the patrol, the studies had to be fitted into the tight schedule of submarine operations. Despite these difficulties, careful collection of samples and meticulous performance of tests produced good data. The staff of the Environmental Physiology Branch spent a great deal of time and effort in the preparation of these studies and in particular in the analyses of the large number of blood and urine samples taken on patrol.

The patrol studies represent longitudinal studies, since the measurements were made prior to the patrol, during, and post-patrol. Even if the interval between exposure and recovery was sometimes several months, there were no problems in assessing physiological functions, such as respiratory function, because paired data were used.

During the 10 years of the existence of this program, sufficient material has been accumulated to warrant a summary report on the evaluation and interpretation of the submarine studies on hypercapnic effects.

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*Braithwaite, Covington, Foster, Gortner, Gude, Harrison, Hughes, Kingsbury, McCluggage, Mendelson, Peck, Rodenbaugh, and Schwartz, in connection with the task titled "Effect of exposure to the total submarine atmosphere and various work rest schedules on physiological functions" assigned to the Environmental Physiology Branch of the Laboratory.

Methods used during patrols and in laboratory investigations

Conventional lung function tests during patrols were performed with a 9-liter Collins spirometer. Standard forced vital capacity maneuvers were performed three times and the highest value of the three trials was selected.

Measurements of physiological dead space aboard submarines (Gude and Schaefer 1969) were carried out as follows: after the subject accustomed himself to a two-way inspiration/expiration valve with a pliable rubber mouthpiece, a 100-liter Douglas bag was attached to collect any expired air over a 10-min period, during which the average respirations per minute were noted.

The volume of the expired air was measured with a dry gas meter and the expired air CO_2 concentration was determined with an infrared CO_2 analyzer (Beckman LB-1).

The 10-min volume of collected expired air was corrected to Body Temperature Pressure Saturated (BTPS) conditions, and the average tidal volume was determined by dividing the 10-min corrected expired volume by the total number of respirations over the 10-min period.

The actual blood pH values and the blood P_{CO_2} values were determined on arterialized capillary blood samples using an ultramicro pH/blood gas analyzer (113-S1, Instrumentation Laboratory, Inc.). Gambino (1959) has shown that arterialized capillary blood can be substituted for the arterial blood sample because it gives equivalent results.

Carbon dioxide tolerance curves were obtained during a patrol by Kingsbury in 1970; the results of that study are presented in this paper. Subjects were breathing ambient air or 5% CO_2 for 10 min through a respiratory valve. Expired air was collected in a Douglas bag during the last three minutes of breathing, and the volume was subsequently measured with a dry gas meter. End-tidal CO_2 was recorded with a Beckman Model LB1 CO_2 analyzer, tapping sample gas from near the mouthpiece. The CO_2 meter was calibrated immediately before measurement, using pure nitrogen for zero and 7.1% CO_2 for the calibration gas. Values of expired volumes were corrected to BTPS.

Blood gas analysis during patrol. Arterialized capillary samples were obtained by digital puncture of siliconized skin after a hand soak of five minutes in water at a temperature of 45°C (Peck 1971). All venous samples were collected in heparinized glass vacuum tubes. After measurements of pH, P_{CO_2}, and P_{O_2} were completed, venous samples were immediately centrifuged at 4000 rpm. The plasma was stored at – 15°C in capped syringes. The measurements of pH, P_{CO_2}, and P_{O_2} were made with an ultramicro pH/blood gas analyzer (Instrumentation Laboratory Model 113-Sl). Blood bicarbonate values were calculated using a standard nomogram.

Collection and storage of plasma and red cell samples during patrols for subsequent analysis at the Laboratory. Venous samples were collected in 7-ml heparinized Vacutainers, spun down immediately, and separated anaerobically. The separated plasma and red cell fractions were then anaerobically transferred to fill a 2-ml test tube completely and frozen at −15°C. Twenty-four hour urine samples were collected, under oil, throughout the patrol and during control and recovery periods. At the end of each 24-h period, the urine volume was measured and a 10% aliquot of the samples was frozen at −15°C. No preservative was used. Analyses of pH and P_{CO_2} were made on a pH blood gas analyzer (I.L. 113-S1, Instrumentation Laboratory, Inc.), Na and K were analyzed on a flame photometer (I.L. 343, Instrumentation Laboratory, Inc.), and Cl with a chloridometer.

Fractional gastric analysis. Patrol samples were taken after 8 days, 23 days, and 54 days (Foster 1969). The fifth set of samples in the study was obtained during the post-patrol period one month after the exposure to elevated atmospheric CO_2 had ended.

All the gastric aspirate samples were taken from fasting subjects. To facilitate the procedure, before introducing a nasogastric tube, each man was given 10 ml of viscous xylocaine with which to gargle. Once the tube was in the stomach, a sample of the gastric content was collected over the next 30 to 45 min. A fractional gastric analysis was performed on 10 ml of the collected material after it had been filtered and the mucus removed. Free and total acidity determinations were made by titration using 0.1 normal NaOH, Topfer reagent (end point pH 2.9–4.0) and phenophthalein (end point pH 8.5). With this method, acid determinations are measured in degree units.

Data evaluation

The physiological functions that were studied undergo circadian cycles. Since many of the crew members were on different work schedules, it was difficult to take measurements of all subjects at the same time after awakening. If this is not taken into account, small but significant changes could be easily masked by circadian cycles, e.g., in acid-base balance studies.

For the statistical analysis, the paired *t*-test was used for a comparison of control data taken prior to the patrol with data obtained during and after patrol. Differences with $P \leq 0.05$ were considered significant.

The relevant variables in the submarine atmosphere

In studying the effect of prolonged exposure to the submarine atmosphere, we are dealing with a multi-factor problem and obviously cannot think in terms of a single-agent cause/effect relationship.

The contaminants in the submarine atmosphere that might affect respiration are listed in Table 1.

Carbon dioxide is the most important factor because it is present in a concentration that has a direct effect on respiration. In fact, it is the only contaminant that is generally on the average of twice that of the listed 90-day Threshold Limit Value (TLV) of 0.5% or 3.8 mmHg; this is so because of the limitations of the on-board scrubber system. All the other variables, such as CO, Freon 14, Freon 12, aerosols, and ions, are present in concentrations below the level at which effects on respiration can be expected (Motley and Kunzman 1958; Anderson and Ramskill 1960; Ramskill 1961; Rodenbaugh 1967; Maumus 1967; Harrison 1968).

Respiration was selected as a target function because of evidence indicating the CO_2 at the low levels found in submarines affects respiration. Another reason for emphasizing respiratory studies was given by the findings of Wilken (1969) showing that respiratory disease is the most prevalent internal medicine problem in submarine cases, followed closely by gastrointestinal diseases (Table 2). This determination was based on the number of sick days. Moreover, upper respiratory infections cause the greatest number of "sick calls," an evaluation category separate from "sick days." Frequently, between 70–90% of the crew made sick calls because of respiratory symptoms, according to Wilken's study.

Methods of presentation of results of physiological studies on submarines

Since CO_2 is the most important contaminant in the submarine atmosphere, amounting on the average to approximately 1% during the period in which most of these studies were carried

TABLE 1

LIMITS, OBSERVED VALUES, AND LEVELS OF ATMOSPHERIC CONTAMINANTS THAT AFFECT RESPIRATORY FUNCTION

Contaminant	90-day Limit TLVs	Frequently Observed Average Values	Levels at Which Respiratory Functions are Affected	Effect
CO_2	3.8 mmHg	6–7 mmHg	6–7 mmHg	Ventilation (Covington, cited in this paper); Dead Space (Gude and Schaefer 1969)
CO	25 ppm	20–30 ppm	500 ppm (Motley and Kunzman 1958)	Increase in Airway Resistance
Freon 114	200 ppm	10–25 ppm −54% Time 25–50 ppm −10% (Rodenbaugh 1967)	850–950 ppm for 1 h; case of bronchitis on submarine (Harrison 1968)	
Freon 12	200 ppm	Average 7 ppm (Rodenbaugh 1967)	No effect on respiration in observed range	
Aerosols	0.3 mg/liter	0.3–0.4 mg/liter (Ramskill 1961; Maumus 1967)	No effect on respiration in observed range	
Ions	—	Average concentration ~ 600(+), 300 (−) ions/cc (occasional surges) (Ramskill 1961; Maumus 1967)	No effect on respiratory function in humans in observed range	(Schaefer, unpublished observations)

In the concentrations observed on nuclear-powered submarines, only CO_2 has a direct effect on respiratory function.

TABLE 2
GENERAL MEDICAL CASES RESULTING IN SICK DAYS ON PATROL

Condition	No. Cases	Total Sick Days
Respiratory	121	480
Gastrointestinal	119	197
Cardiovascular	9	45
Infectious Hepatitis	7	187
Infectious Mononucleosis	18	162
Influenza	74	160
All Other	53	251

Review of 360 patrols, 1963–1967 (Wilken 1969).

out, a comparison was made of the results of patrol studies and the effects of prolonged exposure to 1.5% CO_2 in 21% O_2 (a laboratory simulation study). There was rather close agreement between the effects of prolonged exposure to 1.5% CO_2 and those of the 1% CO_2 submarine atmosphere in regard to: (1) respiration; (2) acid-base balance; (3) electrolyte exchange; and (4) calcium metabolism.

RESULTS

Respiration

Table 3 shows a comparison of ventilatory changes measured under both conditions. Exposure to 1.5% CO_2 produced a consistent increase in respiratory minute volume of 39% and 37% in the two periods (1–24 days and 25–42 days), based on an increase in tidal volume (Schaefer, Hastings, Carey, and Nichols 1963a). Similar but somewhat larger increases in respiratory minute volume (40 and 52%) were observed during the corresponding periods of patrols, although the ambient CO_2 concentrations were lower (Covington data, cited in this paper).

During exposure to 1.5% CO_2, vital capacity showed a decreasing trend, which was more pronounced in the measurements obtained on a patrol after 2, 5, and 8 weeks. The physiological changes in respiratory functions observed during patrols returned to normal during the recovery period ashore.

In Table 4, results of physiological dead space determinations made under the two conditions are compared. Again, there is remarkably close agreement. An increase of about 60% in the physiological dead space was found under both conditions (Schaefer et al. 1963a; Gude and Schaefer 1969).

Figure 1 presents CO_2 tolerance curves obtained during patrol on six subjects who were exposed to an average CO_2 concentration of 1% CO_2. Tests were performed prior to the patrol and on two occasions during the patrol. Inhalation of 5% CO_2 during the patrol produced a significant elevation of both end-tidal CO_2 and minute ventilation compared to similar data obtained during the control period. The rise in end-tidal P_{CO_2} was relatively larger than that of ventilation, resulting in decreased slope of the CO_2 tolerance curve. These findings agree with those obtained during a laboratory experiment, in which subjects were exposed to 1.5% CO_2 for prolonged periods. Figure 2 shows a decreased slope of the average CO_2 tolerance curves

TABLE 3

EFFECT OF PROLONGED EXPOSURE TO 1.5% CO_2, 21% O_2 (LABORATORY) AND 0.8–1% CO_2 (PATROL) ON RESPIRATION

		Respiratory Minute Volume, Liters, BTPS	Percent Change	Respiratory Rate, breaths/min	Percent Change	Tidal Volume, Liters, BTPS	Percent Change	Vital Capacity, Liters, BTPS	Percent Change
				Exposure to 1.5% CO_2 (21% O_2)					
Control Period	Mean	5.32		9.8		0.603		4.56	
	SD	0.12		2.6		0.123		0.76	
	n	(20)		(20)		(20)		(20)	
On 1.5% CO_2	Mean	7.40	+39%	11.4*	+16%	0.704*	+17%	4.47	−2.0%
	SD	0.18		2.0		0.138		0.69	
1–23 Days	*n*	(20)		(20)		(20)		(20)	
24–42 Days	Mean	7.26	+37%	10.5	+ 7%	0.714	+18%	4.52	−1%
	SD	0.27		1.7		0.134		0.65	
	n	(20)		(20)				(20)	
				Exposure to 0.8–1.0% CO_2					
Control Period on Air	Mean	8.03		12.0		0.74		5.42	
	SD	0.40		0.3		0.04		0.37	
	n	(10)		(10)		(10)		(10)	
On 0.8–1% CO_2 14 Days	Mean	11.22*	+40%	12.1	—	1.08	+48%	5.34	−1.5%
	SD	0.6		0.2		0.05		0.33	
	n	(10)		(10)		(10)		(10)	
35 Days	Mean	12.2*	+52%	11.5	−5.8%	1.16	+57%	5.25	−3.1%
	SD	0.8		0.3		0.06		0.32	
	n	(10)		(10)		(10)		(10)	
56 Days	Mean	13.1*	+63%	12.0	—	1.20*	+62%	5.31	−2.1%
	SD	0.8		0.2		0.05		0.23	
	n	(10)		(10)		(10)		(10)	

P <0.05 or better.

TABLE 4
EFFECT OF PROLONGED EXPOSURE TO 1.5% CO_2 AND 0.8–0.9% CO_2 ON PHYSIOLOGICAL DEAD SPACE

		% Change			% Change
Control period on Air n = 10	169±21		Control period on Air n = 6	206±24	
On 1.5% CO_2 in 21% O_2; 40 Days' Exposure n = 9	273±82	+62%*	On 0.9% CO_2 in 20–21% O_2; 20 Days' Exposure n = 6	367±49	+56%
4 Weeks' Recovery on Air n =8	174±25	+ 3%*	8 Weeks' Recovery on Air = Control Period n = 6	216±40	
			On 0.8% CO_2 in 20–21% O_2 20 Days' Exposure**	369±42	+59%*

Values are means ± SD; *difference significant at 5% level or better;** exposure to 0.8% CO_2 during second patrol.

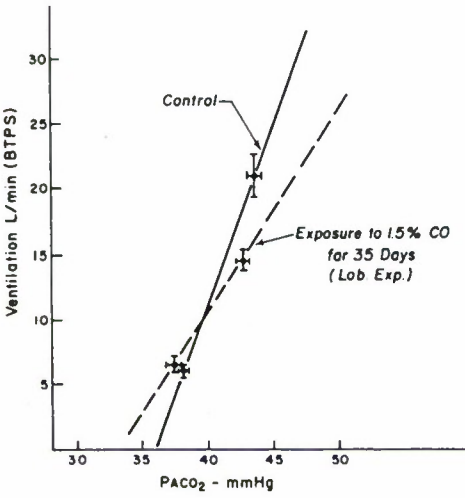

Fig. 1. Ventilation (BTPS) plotted against end-tidal P_{CO_2}. Decrease in slope of CO_2 tolerance curve obtained during patrol on 6 subjects after 14–18 days and 25–32 days of exposure to an average CO_2 concentration of 1% CO_2.

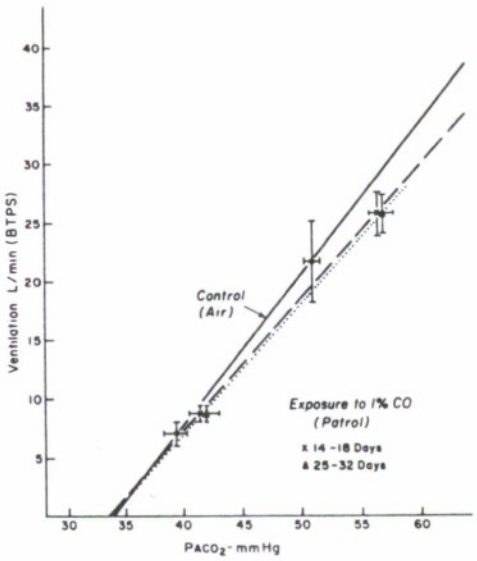

Fig. 2. Ventilation (BTPS) plotted against end-tidal P_{CO_2}. Decrease in slope of CO_2 tolerance curves after 35 days' exposure to 1.5% CO_2.

determined in 21 subjects after 35 days of exposure to 1.5% CO_2. However, this change is based mainly on a marked decline in ventilatory response; the end-tidal CO_2 tension changed very little.

Acid-base balance and electrolytes

Patrol studies carried out at a 1% CO_2 level demonstrated periods of a slight respiratory acidosis, indicated by slightly elevated P_{CO_2}, lowered pH, increased bicarbonate, and decreased plasma chloride (Gude and Schaefer 1969; Peck 1971; Gortner, Messier, Heyder, and Schaefer 1971; Messier, Heyder, Braithwaite, McCluggage, Peck, and Schaefer 1979), as well as periods of predominant metabolic acidosis, in which bicarbonate was lower.

The time course of pH changes in the arterialized capillary blood and in venous blood determined on 15 subjects during a 64-day patrol using an ultramicro pH blood analyzer (Instrumentation Laboratory Model 113-S1) (Peck 1971) is shown in Fig. 3, together with the P_{CO_2} level of the atmosphere. Because of the different watch schedules of the naval personnel involved in the studies, the samples were collected at times that varied greatly with regard to the length of the awake period (30 min to 24 h). To eliminate the known effects of circadian cycles on pH, the subjects were divided into two groups. In Group A, the blood samples were taken within four hours of awakening, and in Group B, longer than four hours after awakening. The measurements of pH in the arterialized capillary blood and in venous blood were made independent of each other on different blood samples. The time course of pH changes in arterialized capillary blood and venous blood correspond with each other, and so do those of the two subject groups. The pH falls during the first two weeks and subsequently rises to normal values. From the 42nd day on, another decline in pH occurs. Similar changes, shown in Fig. 4, were observed during the time course of plasma bicarbonate in arterialized capillary and venous blood. The significant decreases in bicarbonate during the third week and again during the seventh and eighth weeks of the patrol indicate the development of metabolic acidosis superimposed on the CO_2-induced respiratory acidosis. An increase in bicarbonate normally associated with a respiratory acidosis is observed during the first days of the patrol and after about four weeks.

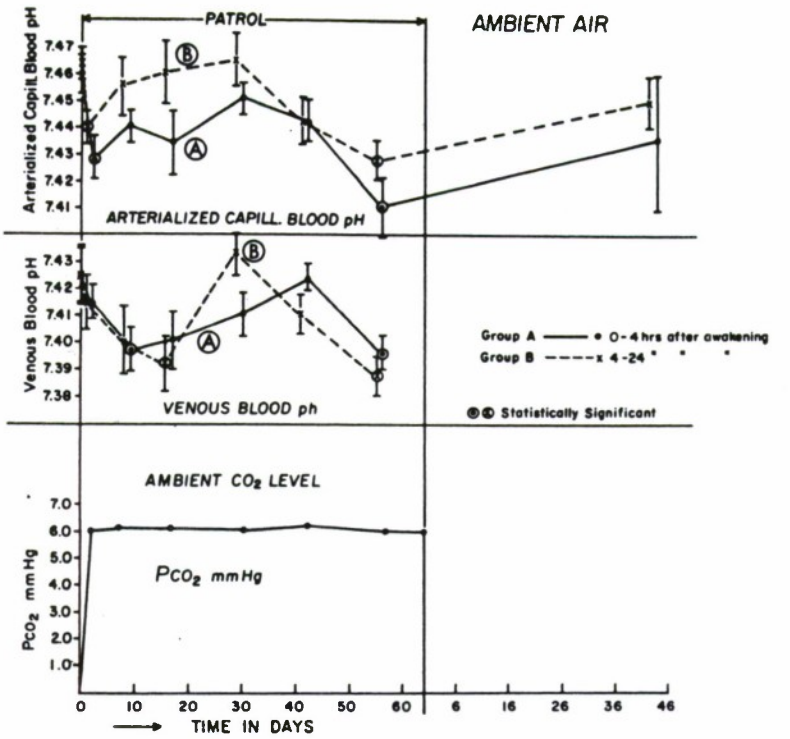

Fig. 3. Time course of pH in arterialized capillary and venous blood during patrol. Average ambient P_{CO_2} level 6 mmHg (Peck 1971: 15 subjects, two groups; group A: sample taken 0–4 h after awakening; B: samples taken 4–24 h after awakening).

Changes in blood CO_2 tension observed during patrol in arterialized and venous blood are presented in Fig. 5. Again, there are cyclic changes—an increase of about 2 mmHg during the first week, a subsequent fall of about 2 mmHg below control levels during the third week, followed by another rise and another fall. In other patrol studies in which a much more limited amount of data was obtained, a rise of about 4 mmHg P_{CO_2} in arterialized blood (10 subjects) was observed by Gude and Schaefer (1969) after 20 days of exposure to 1% CO_2. Schwartz (1969), using the Hackney-Collier CO_2 rebreathing method to estimate arterial P_{CO_2}, found an increase averaging 4 mmHg in eight subjects after 21 days' exposure to 1% CO_2; this agrees with the findings of Gude and Schaefer (1969). After 42 days of exposure to 1% CO_2, the estimated arterial blood P_{CO_2} was only 1.4 mmHg above control levels.

Data on pH, P_{CO_2}, and bicarbonate obtained in three patrol studies and two laboratory studies are shown in Fig. 6. The average ambient CO_2 ranged from 0.85%–1% CO_2 in the patrol studies and 1%–1.5% CO_2 in the laboratory simulation experiments. In all experiments, cyclic changes in pH are evident. The time periods of these cycles are about 20 days for the initial fall, subsequent rise, and second fall in pH. These three periods, which are also reflected in P_{CO_2} and bicarbonate, have been classified as a sequence of metabolic acidosis, respiratory acidosis, and metabolic acidosis. The longest experiment, of 90 days' duration, in which four subjects were exposed to 1% CO_2 (McDonnell 1971; Messier, Heyder, and Schaefer 1971) showed as many as four cycles of pH changes.

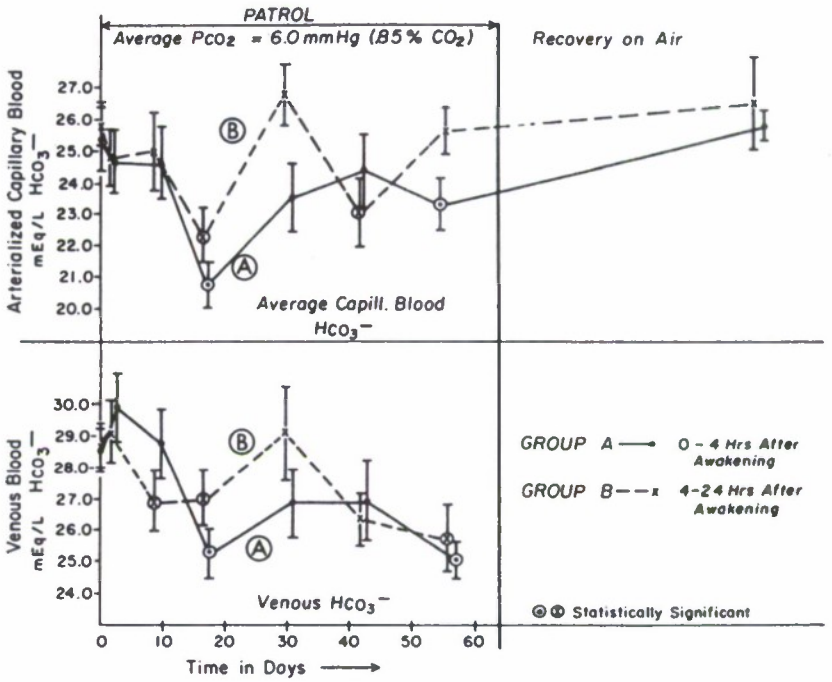

Fig. 4. Time course of bicarbonate in arterialized capillary blood and venous blood during patrol. Average ambient P_{CO_2} level = 6 mmHg: 15 subjects, two groups; group A, samples taken 0–4 h after awakening; B, 4–24 h after awakening (Peck 1971).

Data on plasma chloride collected on patrols are very limited. A significant decrease of plasma chloride during prolonged exposure to 0.9%–1% CO_2 on patrols was observed by Gortner et al. (1971) on Day 42, by Mendelson (cited by Gortner et al. (1971)) on Day 40, and by Messier et al. (1979) on Days 36 and 51. The fall in chloride occurs during the periods of respiratory acidosis and appears to correspond with the rise in bicarbonate.

In three of the studies that provided information on the acid-base status exhibited in Fig. 6, plasma calcium was measured. These data are exhibited in Fig. 7, together with those obtained during a patrol study by Gray, Morris, and Brooks (1973). In this figure, 20-day periods have been marked in the same manner as in Fig. 6. In most cases there is a fall of plasma calcium during the first 20 days, followed by a marked rise during the second 20-day period. The peaks of plasma calcium center around the 40th day, with the exception of one on Day 51. Between 40 and 60 days of exposure, there is again a decline in plasma calcium. The clearly pronounced cycles in plasma calcium follow the cycles in acid-base balance shown in Fig. 6.

Data on urinary excretion and urine volume collected in the same experiments are exhibited in Fig. 8. Cyclic changes in calcium excretion can be seen in every experiment, although the time periods do not correspond with each other in all cases. A more detailed presentation of data on urinary pH, titratable acidity, and calcium and phosphorus excretion collected during the 90-day experiment (1% CO_2) is given in Fig. 9. Cyclic pH changes occur in 20-day periods and correspond approximately to the cycles in calcium and phosphorus excretion. During the period in which the pH rises, urinary calcium and phosphorus excretion increase. Titratable acidity is higher during the first part of the experiment and lower during the second part and

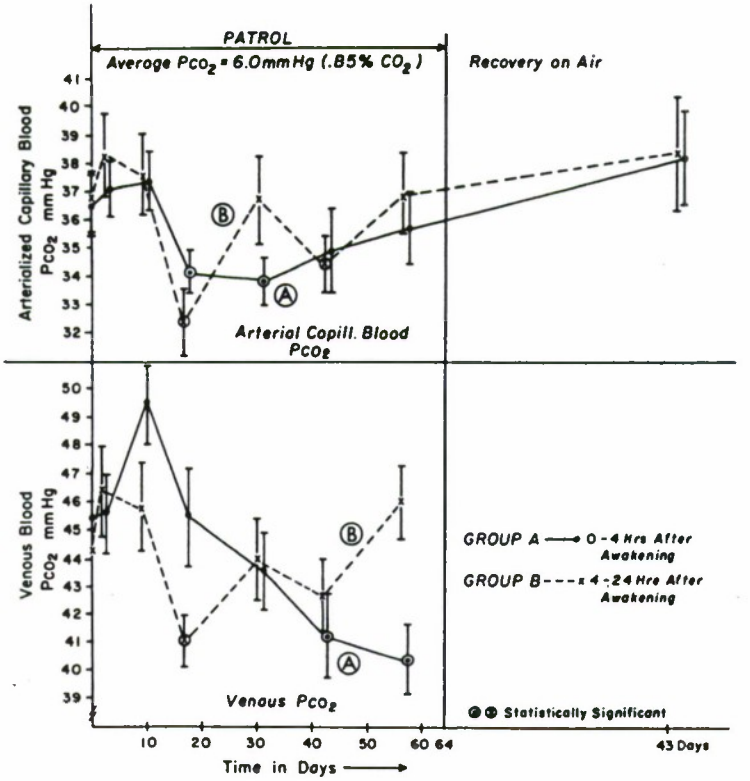

Fig. 5. Time course of CO_2 tension in arterialized capillary blood and venous blood during patrol. Average ambient Pco_2 level = 6 mmHg; 15 subjects, two groups; A, samples taken 0–4 h after awakening; B, 4–24 h after awakening.

does not show a clearly pronounced relationship to calcium excretion. However, in a British patrol experiment in which the ambient CO_2 level was 0.7% CO_2, a slight rise in calcium excretion at the 20th day of exposure was observed, which corresponded exactly with the rise in acid excretion shown in Fig. 10 (Gray et al. 1973). Data on urinary electrolyte excretion measured in the same experiment are plotted with the plasma calcium, magnesium, and phosphorus data collected simultaneously. Both plasma calcium and phosphorus show two peaks, on Day 5 and Day 40. Magnesium also exhibits an early peak on Day 5. However, there is no second peak on the 40th day. Urinary excretion of calcium, phosphorus, and magnesium do not reflect any of the peak plasma levels of these electrolytes. The findings of Gray et al. (1973) have been summarized and reproduced in this paper because they represent the most comprehensive published blood and urine data collected in human subjects exposed to low levels of CO_2 on patrols and correspond with findings on plasma, calcium, and phosphorus obtained in guinea pigs exposed to 1% CO_2 for 8 weeks, in which bone electrolytes were also measured (Schaefer, Pasquale, Messier, and Niemoeller 1979b). These findings will be discussed later.

Plasma electrolytes

Measurements of plasma Na concentrations during chronic low level hypercapnia produced equivocal results. During exposure to 1.5% CO_2, Schaefer, Nichols, and Carey (1963b) ob-

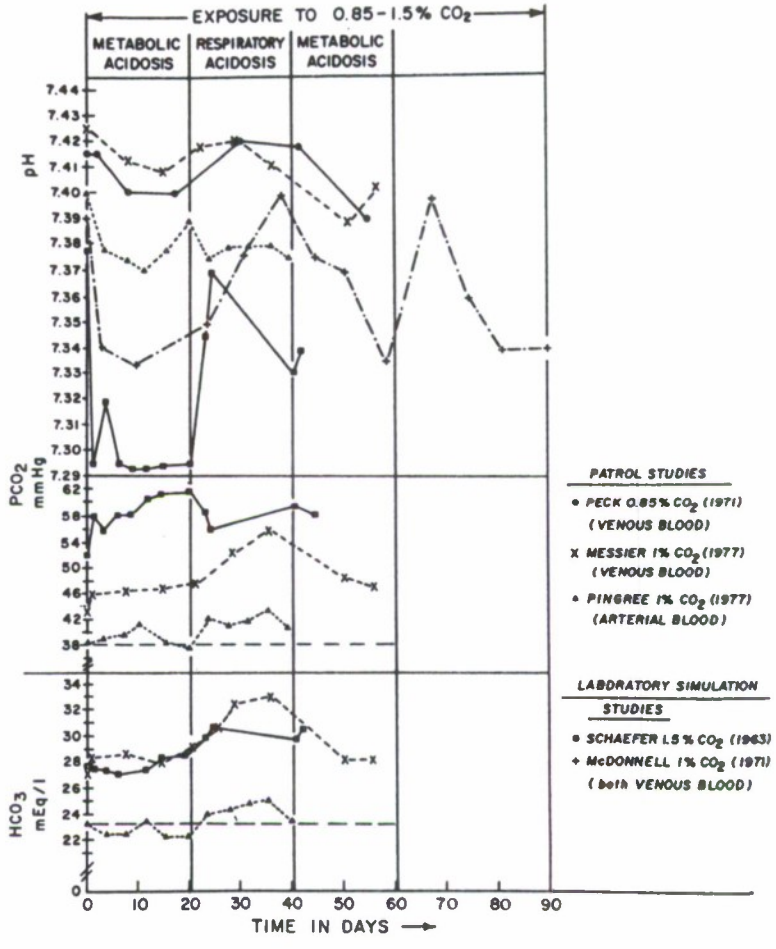

Fig. 6. Time course of pH, blood P_{CO_2} and bicarbonate during patrols (ambient CO_2 levels 0.85–1% CO_2) and during laboratory simulation tests in which subjects were exposed to 1.5% CO_2 for 43 days and 1% CO_2 for 90 days.

served an increase of 2 mEq during the first 24 days, and a subsequent return to control values. Messier et al. (1971) observed a decrease of 4 mEq during the first 24 days of exposure to 1% CO_2. In two patrol studies in which the combined CO_2 concentrations varied between 0.85–0.9% CO_2, a trend toward reduction in plasma Na values was seen (Peck 1971; Gortner et al. 1971). In another patrol study (Messier et al. 1979) an increase of plasma Na was observed.

Data on plasma K measured during laboratory and patrol studies are listed in Table 5. During exposure to 1.5% CO_2, plasma K content of the arterial blood showed a significant decrease. A decrease in plasma K was also observed during the first part of the McDonnell study and in three patrol studies in which plasma K was measured.

Red cell electrolytes

Red cell sodium increased and red cell potassium decreased both during exposure to 1.5% CO_2 and exposure to 1% CO_2 submarine atmosphere, as shown in Tables 6 and 7 (Schaefer, Nichols, and Carey 1964; Gortner et al. 1971; Messier et al. 1979). Similar changes occurred in

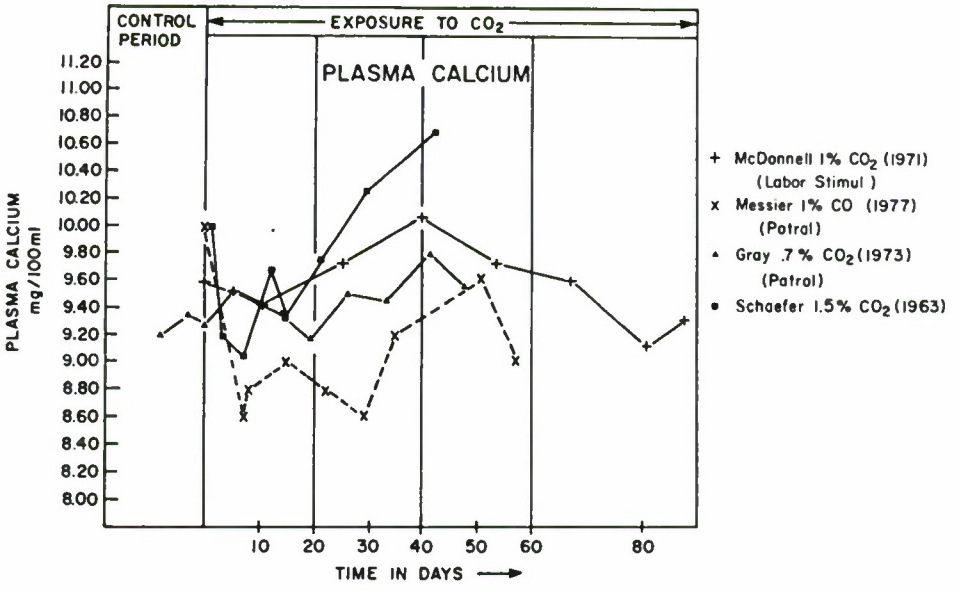

Fig. 7. Summary of plasma calcium data obtained during patrols with ambient CO_2 concentrations ranging from 0.7% CO_2 (Gray et al. 1973) to 1% CO_2 (Messier et al. 1979), and laboratory experiments with ambient CO_2 concentrations ranging from 1% CO_2 (McDonnell 1971) to 1.5% CO_2 (Schaefer 1963b).

red cell calcium during prolonged exposure to 1.5% CO_2 (laboratory experiment) and 1% CO_2 during patrols (Messier et al. 1979). In both cases there were increases in red cell calcium.

Saliva electrolytes

Studies by Hughes (1969) of salivary CO_2 and electrolyte excretion during exposure to a 1.2% CO_2 atmosphere on a patrol showed an increase of CO_2 content and corresponding decrease of chloride, while salivary flow rate remained at the control level. Calcium concentration decreased, which agrees with findings showing a decrease in serum calcium during three patrols (Messier et al. 1979) (Table 8). These saliva studies give additional evidence of the existence of a CO_2-induced acidosis on patrols.

Gastric secretion

Since many sailors complain during patrol about pyrosis symptoms, which are associated with increased gastric activity, a study was made of gastric acidity on five volunteer crew members during a patrol. All five subjects had above-normal total gastric acidity values during patrol compared with pre- or post-patrol levels (Table 9) (Foster 1969).

DISCUSSION

Respiration

The patrol study by Covington (cited in this paper) showed that prolonged exposure to CO_2 concentrations in the range of 0.85–1% CO_2 produces a continuous stimulation of respiration,

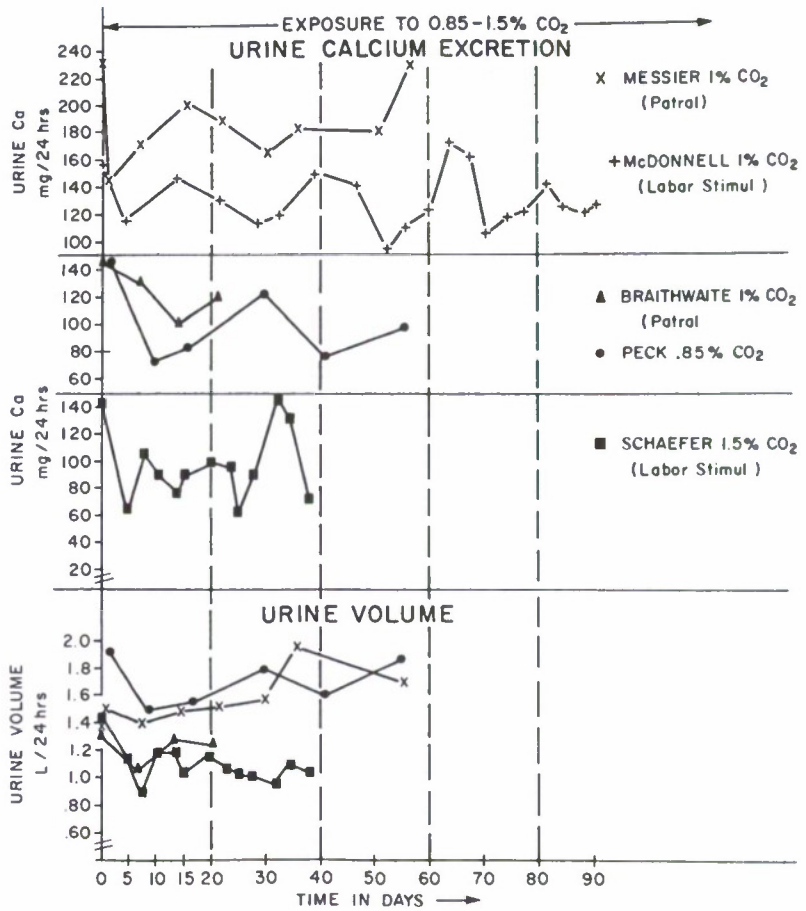

Fig. 8. Summary of urinary calcium excretion and urine volume data collected during patrols (ambient CO_2 levels 0.85%–1% CO_2) and laboratory experiments (ambient CO_2 concentrations 1% CO_2–1.5% CO_2).

demonstrated by the consistent increase in ventilation caused by an increased tidal volume throughout the exposure period.

In other patrol studies in which the ambient CO_2 concentration was 1%, a smaller increase in respiratory minute volume was observed (Pingree 1977; Kingsbury (cited in this paper)).

A summary of the effects of prolonged exposure to low CO_2 concentrations in the range of 0.8–2% CO_2 is presented in Table 10. The first two laboratory experiments with 2% and 1.5% CO_2 show very similar changes in end-tidal CO_2 tension ($P_{A_{CO_2}}$) and respiratory minute volume. Respiratory minute volume shows a modest decrease in the later part of the exposure period, while $P_{A_{CO_2}}$ remains essentially at the same level. In the three patrol studies listed, the ambient CO_2 concentrations ranged from 0.8–1% CO_2. A marked increase in ventilation was found during the first part of the exposure period in all three patrols, and was associated with very little or no decrease in $P_{A_{CO_2}}$. During the later portion of the patrol period, respiratory minute volume declined but remained above the control level in two studies; it fell 22% below control data in the study of Pingree (1977). However, the control respiratory minute volume reported by Pingree of 11.5 liter/min is much too high for resting conditions. It is therefore most likely that the subjects were not sufficiently trained and that they therefore

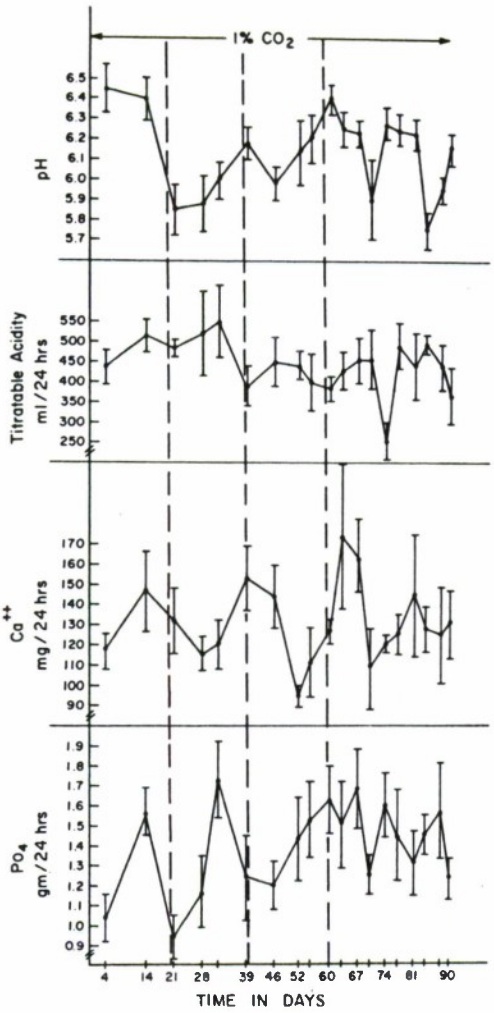

Fig. 9. Urine pH, titratable acidity, urine calcium, and phosphorus excretion during prolonged exposure to 1% CO_2 (4 subjects) (McDonnell 1971).

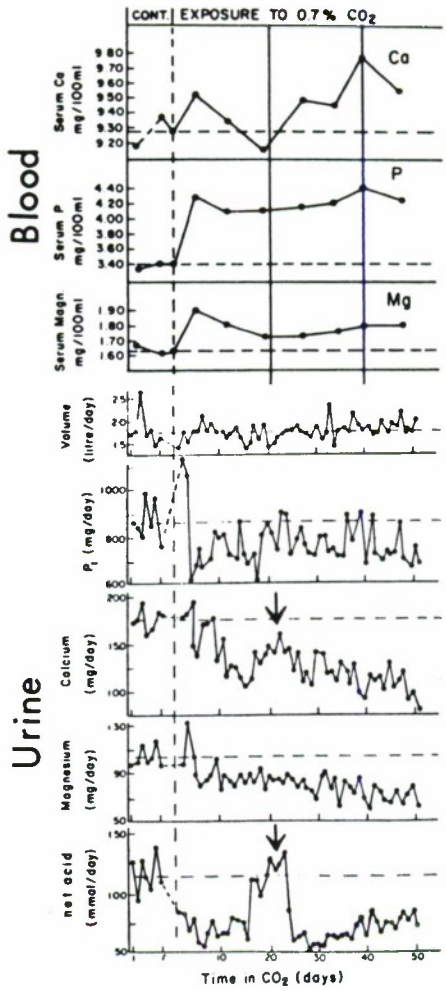

Fig. 10. Data on serum calcium, phosphorus and magnesium, and urinary calcium, magnesium, phosphorus, and acid excretion collected during prolonged exposure to 0.7% CO_2 on patrol (Gray et al. 1973).

hyperventilated. If one assumes a control respiratory minute volume of 8.0 liter/min, the decrease in the respiratory minute volume after the rise in the initial exposure period would bring the values down approximately to control values and not below. The importance of these studies (cited in Table 11) is the evidence for a continuous stimulation of respiration during long periods associated with such small increases in $P_{A_{CO_2}}$. In the later part of the studies, the hyperventilation effect declines. It seems to disappear altogether, according to the study of Pingree (1977). This agrees with findings in a recent British simulation study in which subjects were exposed for 36 days to 0.5% CO_2 and ventilation increased only during the first five days (Davies, Smith, Leitch, Morris, and Gray 1976). During exposure to the submarine atmosphere, factors other than CO_2 might increase ventilation. Aerosols and ions may interact with

TABLE 5

EFFECT OF PROLONGED EXPOSURE TO LOW LEVELS OF CO_2 ON PLASMA K CONCENTRATIONS

Experiment (Schaefer), $n=10$; arterial blood, on 1.5%CO_2	K, mEq/liter mean ± SEM 4.77±1.5	Patrol (Messier), $n=7$; venous blood, on 1% CO_2	K, mEq/liter means ± SEM 4.3±0.07	Patrol (Peck); $n=15$; venous blood, on 0.85% CO_2	K, mEq/liter means ± SEM 5.9±0.08	Patrol (Gortner), $n=12$; venous blood, on 0.9% CO_2	K, mEq/liter mean ± SEM 3.9±0.0	Experiment (McDonnell), $n=4$; venous blood, on 1% CO_2	K, mEq/liter means ± SEM 4.2±0.1
35–41 days	4.41 ±0.37*	Day 7	4.4 ±0.09	Day 2	3.9 ±0.07	Day 7	3.6 ±0.12*	Days 1–24 (16)	3.81 ±0.1*
Recovery on Air (9 days)	4.08 ±0.24*	Day 8	4.1 ±0.12	Day 9	3.7 ±0.07	Day 21	3.8 ±0.16	Days 25–45 (12)	4.0 ±0.1
		Day 15	4.1 ±0.19	Day 17	3.4 ±0.10*	Day 42	3.8 ±0.17	Days 49–90 (20)	4.2 ±0.1
Recovery on Air (4 Weeks)	4.32 ±0.37*	Day 22	3.9 ±0.06*	Day 30	3.8 ±0.04	Day 63 + Recovery 3 days on air	3.9 ±0.24	Post exposure Recovery 1–25 Days	
		Day 29	3.7 ±0.07*	Day 56	3.7 ±0.08				4.2 ±0.2
		Day 30	3.7 ±0.07*						
		Day 51	4.0 ±0.08*						
		Day 57	3.8 ±0.06*						

*Difference significant at the 5% level or better; control periods on air.

TABLE 6
EFFECT OF PROLONGED EXPOSURE TO 1.5% CO_2, 21% O_2 ON PLASMA AND RED CELL Na AND K CONCENTRATIONS

		Plasma		Red Cells	
		Na, mEq/liter	K, mEq/liter	Na, mEq/liter	K, mEq/liter
Control Period on Air	Mean	141.4	4.77	13.5	86.0
	SD	±2.9	±0.15	±4.5	±4.5
	n	(9)	(10)	(10)	(10)
On 1.5% CO_2 35–41 Days	Mean	141.2	4.41*	21.6*	78.9*
	SD	±0.6	±0.37	±4.8	±4.4
	n	(10)	(10)	(9)	(9)
8–9 Days Recovery on Air	Mean	140.0	4.08*	24.4*	76.2*
	SD	±1.4	±0.24	±4.9	±4.7
	n	(8)	(9)	(8)	(9)
4 Weeks Recovery on Air	Mean	142.0	4.32*	12.8	79.9*
	SD	±4.1	±0.37	±6.9	±4.2
	n	(10)	(10)	(8)	(10)

*Difference significant at the 5% level or better. Data from Schaefer et al. 1964.

TABLE 7
EFFECT OF PROLONGED EXPOSURE TO 1% CO_2 DURING PATROL ON RED CELL Na AND K CONCENTRATIONS

		Plasma		Red Cells	
		Na, mEq/liter	K, mEq/liter	Na, mEq/liter	K, mEq/liter
Control Period on Air	Mean	131.2	4.3	11.1	72.2
	SEM	1.5	0.07	0.2	1.3
	n	(7)	(7)	(7)	(7)
On 1% CO_2 8 Days	Mean	136.3*	4.1	17.4*	67.1*
	SEM	0.9	0.12	1.5	1.7
	n	(10)	(10)	(10)	(10)
22 Days	Mean	137.3*	3.9*	16.0*	68.1*
	SEM	2.1	0.06	1.1	0.5
	n	(10)	(10)	(10)	(10)
36 Days	Mean	136.0*	3.7*	19.8*	65.1*
	SEM	0.7	0.08	0.8	0.7
	n	(9)	(9)	(9)	(9)
51 Days	Mean	136.9*	3.8*	17.5*	67.5*
	SEM	0.4	0.06	0.7	0.6
	n	(10)	(10)	(10)	(10)

*Difference significant at the 5% level or better. Data from Messier et al. 1979.

TABLE 8
ANALYSIS OF SALIVARY COMPONENTS DURING AN FBM PATROL

	Flow, ml/5 min	CO_2, vol%	Na^+, mEq/liter	K^+, mEq/liter	Ca^+, mg/100 ml	P_i, mg/100 ml	Cl^-, mEq/liter
Pre-patrol	4.7	55.87	24.85	18.95	4.7	10.71	37.76
	0.49	6.73	2.73	0.99	0.22	0.41	4.02
6th week of patrol	4.6	66.13	22.8	19.63	4.23	14.83	30.91
	0.34	7.63	3.44	1.07	0.33	1.81	3.32

Values are means, with SEMs below; $n = 10$. Data derived from Hughes 1969.

CO_2. This would explain the fact that in the study of Covington, a lower concentration of CO_2 (0.8–1%) produced a higher increase in ventilation (Table 3) than exposure to 1.5% CO_2, and showed an even further increase in the later part of the patrol.

Guillerm and Radziszewski (1979), in their excellent study on the effects of prolonged exposure to 2% CO_2 for 30 days, have shown an immediate decrease in CO_2-induced hyperventilation in the period between 2 and 24 h of exposure. They observed a similar decline in hyperventilation during exposure to 3% and 4% CO_2, which was associated with a slight increase in $P_{A_{CO_2}}$. The authors could demonstrate that this initial decline could not have been caused by changes in $P_{A_{CO_2}}$ and arterial pH. Alterations in cerebrospinal fluid pH and peripheral chemoreceptor activity could also be excluded. It was concluded that this early alteration of hyperventilation might be related to a decrease in the respiratory center sensitivity to the P_{CO_2} stimulus. The data published by Guillerm and Radziszewski (1979) showed a second and more pronounced attenuation of the CO_2-induced hyperventilation between the 8th and 15th day of exposure to 2% CO_2, a finding to which the authors made no reference. This decline in respiratory minute volume occurred after a compensation of the acidosis was reached on Day

TABLE 9
EFFECT OF PROLONGED EXPOSURE TO 1% CO_2 ON GASTRIC SECRETION DURING PATROL

Condition	Total Acid, Degrees
Control Period on Air	41.2±3.3
On 1.0% CO_2 for 8 Days	54.2±6.4
23 Days	57.4±11.7
54 Days	64.0±13.4
Post-Patrol 4 Weeks Recovery on Air	32.2±6.1

Values are means ± SEM; $n = 5$. Data derived from Foster 1969.

TABLE 10
EFFECT OF EXPOSURE TO LOW CO_2 CONCENTRATIONS ON $P_{A_{CO_2}}$ AND RESPIRATORY MINUTE VOLUME

Ambient CO_2, %	Duration, Days	n	Control $P_{A_{CO_2}}$, mmHg	CO_2 Exposure, $\Delta P_{A_{CO_2}}$	CO_2 Exposure, $\Delta P_{A_{CO_2}}$	Control Respiratory Minute Volume, BTPS	CO_2 Exposure Respiratory Minute Volume, %	CO_2 Exposure Respiratory Minute Volume, %	References
						Laboratory			
2%	30	6	37.8	1–9 Days +2.5	10–30 Days +2.5	7.8 liter/min	1–9 Days +38%	10–30 Days +30%	Guillerm and Radziszewski (1979)
1.5%	42	20	37.8	1–24 Days +2.4	25–42 Days +2.1	6.1 liter/min	1–24 Days +39%	25–42 Days +37%	Schaefer et al. (1963a)
						Patrol			
0.8–1%	56	10	—	14 Days —	35 Days —	8.03 liter/min	14 Days +40%	35 Days +52%	Covington, cited in Schaefer (1978)
1%	50	5	40.6	14–18 Days —	25–30 Days —	7.5 liter/min	14–18 Days +16%	25–32 Days +9%	Kingsbury, cited in Schaefer (1978)
1%	44	15	38.2	1–4 Days +0.4	16–44 Days +3.1	11.5	1–4 Days +33%	16–44 Days −22%	Pingree (1977)

TABLE 11
SUMMARY OF PHYSIOLOGICAL EFFECTS OF PROLONGED EXPOSURE TO 1% CO_2 IN 20–21% O_2 ON SUBMARINE PATROLS

Respiration	Increase in respiratory minute volume (+40–60%); Increase in tidal volume (+40–60%); Increase in physiological dead space (+50–60%); Decrease in vital capacity (−3%)	Covington 1968 Gude and Schaefer 1969
Acid-Base balance	Respiratory acidosis; Increase in P_{CO_2} and decrease in pH for different periods	Messier et al. 1979; Schwartz 1969; Gortner et al. 1971; Peck 1971
Electrolytes	Decrease of plasma chloride related to acidosis; Red cell Na increase, K decrease Saliva: Increase in CO_2, decrease in Cl	Messier et al. 1979; Mendelson 1969; Hughes 1969
Gastric acid secretion	Increase in total gastric acid (5 subjects) throughout patrol	Foster 1969
Calcium-Phosphorus metabolism	Decrease in plasma calcium; Decrease in urine calcium; Decrease in urine magnesium	Messier et al. 1979
	Red cell calcium increase during patrol	Messier et al. 1979; Braithwaite 1972

15. It is therefore most likely that this second attenuation of the CO_2-induced hyperventilation was related to the compensation of the respiratory acidosis associated with plasma bicarbonate increase (Torino, Goldring, and Heinemann 1974).

In the other hypercapnia studies listed in Table 11, no measurements were made at 2 h of exposure. Consequently, no comparison can be made with the data on 2% CO_2 with regard to the early changes in respiratory minute volume. However, the second decline of the CO_2-induced hyperventilation after 2–4 weeks of exposure is pronounced in all the other studies, with the exception of that of Covington. It should be pointed out that all acid-base balance studies performed during low level chronic hypercapnia showed a phase of compensatory respiratory acidosis during the time in which this later decline in respiratory minute volume was observed (Fig. 6). This supports the conclusion that acid-base changes caused the decreases in ventilation during the later part of the exposure.

The increase in physiological dead space found during patrols agrees with the changes observed during exposure to 1.5% CO_2 (Schaefer et al. 1963a) and seems to indicate a dilating effect of CO_2 on the airways. The significance of these findings can only be assessed through regular pulmonary function studies of submariners in a longitudinal health study.

The observations of Sonnenburg (1965) on the effect of submarine air on ciliary mucus transport should be mentioned. During a routine patrol of an FBM submarine, he measured the ciliary mucus activity in freshly prepared frog esophagus strips exposed to "submarine air" and "surface air." The latter was brought aboard the submarine in cylinders. Results

showed a definite decrement in ciliary activity in those tissues exposed to submarine air. The author suggested that activity of (+)ions, in association with CO_2 and aerosols, might account for these results. Air ions have been measured in FBM submarines and are in general no different from those of the natural atmosphere. Average concentrations for positive and negative ions are less than 1,000/cc, considerably lower than concentrations measured in conventional fleet-type submarines (Schaefer 1961b). Occasionally, ion surges occur and higher concentrations are reached (Ramskill 1961; Maumus 1967). Interaction of aerosols and ions may result in a shift of the spectrum of ions (Schaefer and Dougherty 1961). Respiratory functions were measured in our laboratory in human subjects exposed to (+) and (−) ion concentrations observed on submarines. No effects were observed. However, further studies on the combined effects of aerosols, ions, and low levels of CO_2 would be required to see whether aerosols and ions in the submarine atmosphere interact with ambient CO_2 and influence respiration.

Verzar (1962) has pointed out that condensation nuclei (aerosols) in the closed-space atmosphere might play a role by concentrating toxic trace substances, e.g., organic substances containing acid material, causing them to exceed effective threshold levels. This could occur in the ambient air or in the respiratory tract during normal respiration and could have effects on respiration.

In another study of pulmonary functions carried out by Rodenbaugh (1967) on 135 members of a Polaris crew on patrol, six crew members were found to have definitely abnormal pulmonary functions and several more were borderline cases, as indicated by reduced maximal expiratory flow rates and 1-, 2-, and 3-s vital capacity data. Smokers had significantly lower maximal flow rates than non-smokers.

Rodenbaugh (1967) attempted to compare the 2.5% incidence of abnormal lung functions in his submarine crew with that of an industrial population of males below the age of 40 years; the latter incidence was reported to be 0.5%. However, further studies would be required to establish whether there is a greater incidence of abnormal pulmonary functions in submariners during patrols.

Acid-base balance and electrolytes

The cyclic changes in blood pH, bicarbonate, and P_{CO_2} observed during patrols involving prolonged exposure to 0.85%–1% CO_2 and during laboratory simulation experiments with ambient CO_2 levels of 1.0 to 1.5% CO_2 (Fig. 6) are significant findings. They demonstrate a new phenomenon that has not been described previously. The time course of pH, bicarbonate, and P_{CO_2} shown in Fig. 6 shows alterations between a metabolic acidosis during the first 15–20 days, followed by a respiratory acidosis (20–40 days) and a subsequent metabolic acidosis during the period between 40 and 60 days.

Moreover, Gray et al. (1973) observed cyclic changes in urinary net acid excretion and ammonia excretion during 7 weeks of exposure to 0.7% CO_2 on a submarine. Under these conditions, similar to those reported in this paper, net acid and NH_4 excretion decreased during the first two weeks; this was followed by a rise to control levels that lasted for 10 days (up to Day 24) and a subsequent fall, with no further change, until the end of the exposure.

From what is known about renal regulation during CO_2-induced respiratory acidosis, one would expect that an increased acid load would be met with an increased net acid and ammonia excretion. Recent studies on the mechanism of urinary acidification (Rector 1974) indicate that H^+ secretion plays a major role in both bicarbonate reabsorption and formation of titratable acid and ammonia. For each hydrogen ion excreted in the urine through titratable

acid and NH_4, a newly formed bicarbonate is added to renal venous blood. The fall in blood bicarbonate observed during the first 17 days of exposure to 0.85% CO_2 (Fig. 3) and the failure of the kidney to respond with an increased acid and NH_4 excretion to the acid load during the first two weeks of exposure to 0.7% CO_2 (Gray et al. 1973) indicate that renal defense mechanisms known to operate against respiratory acidosis induced by higher CO_2 concentrations are not brought into play immediately under lower CO_2 concentrations. The rise in blood bicarbonate and the increase in net acid and NH_4 excretion occur, however, after a delay of nearly three weeks. What caused this delay?

Acid-base balance, CO_2 storage, and calcium homeostasis

I am trying to show that this delayed renal response in low level chronic hypercapnia is related to the dominant role of bone buffering in the regulation of acid balance, in particular to the processes of bone CO_2 storage and associated bone calcium changes.

Data on blood calcium obtained during prolonged exposure to 0.70–1.5% CO_2 (Fig. 7) show cycles of 14–20 days, which mirror those for pH exhibited in Fig. 6. Except for an initial peak in the first few days of exposure, plasma calcium falls during the first three weeks and then rises during the period between 20 and 40 days. Urinary calcium excretion is definitely reduced during the first 20-day period in all cases and subsequently shows an increase that may vary in length (Fig. 8). The cycles of increased urinary calcium excretion do not correspond to the peaks of blood calcium but are shifted to a later time that may be related to the interaction of parathyroid hormone and calcitonin, which raise and decrease the threshold of urinary calcium excretion, respectively (Peacock, Robertson, and Nordin 1969; Crumb, Martinez-Muldonado, Eknoyan, and Suki 1974).

The activity of parathyroid hormone and calcitonin was measured in two patrol studies in which the ambient CO_2 concentration was 1% CO_2 (Messier et al. 1979). In one of these studies, a tendency towards an increase was observed after two and three weeks, but the differences were not statistically significant. In the second study, no trends in parathyroid hormone activity (PTH) were observed. No significant changes in calcitonin levels were found; however, it should be remembered that the tests carried out at the Endocrine Laboratory of Mass. General Hospital had about a 15% variability. Under these conditions it is difficult to establish statistically whether minor but physiologically significant increases in PTH activity or calcitonin occurred. Increased levels of PTH have been found to decrease bicarbonate reabsorption and to produce a systematic acidosis (Crumb et al. 1974). Such a condition may have played a role in the development of the acidosis observed during the first three weeks of exposure to increased CO_2 levels.

The CO_2-induced decreases in urinary calcium excretion during the first 2–3 weeks of exposure were in most cases associated with a decrease of serum calcium. This is in agreement with earlier findings of Schaefer and his group (1963b) at a 1.5% CO_2 level. It has been suggested that these changes in calcium metabolism during adaptation to CO_2 mark the deposition of CO_2 in bones (Schaefer et al. 1963b) and that bones play an important role in acid-base regulation. More recent studies carried out by Bursaux and Poyart (1974) have given further support to this view. It was found that bone has a rapidly exchangeable pool of bicarbonate, amounting to about 30% of the total bone CO_2, which equilibrates in a rather short time with the level of CO_2 in the blood. In good agreement with the findings of Bursaux are data obtained by Pellegrino and Biltz (1965) showing that in patients with uremia, 37% of the carbonate of the bones was lost. Furthermore, 5% of the total calcium was used for the buffering of hydrogen ion acidosis.

During exposure to low CO_2 concentrations (between 0.7–1.5% CO_2), the acid load seems so small that the threshold of the kidney's regulation is not reached. The bone apparently is the first line of defense under these conditions. However, the bone's defense is static compared to the dynamic defense of the kidney, and it depends on the capacity of its CO_2 stores, which amounts to 110 liters for a 70-kg man or 80% of total body CO_2 stores (Rahn 1962). When capacity is reached, it can be assumed that CO_2 is released from the bone after about three weeks, in accordance with the long time-constants of the bone CO_2 store (Freeman and Fenn 1953). A CO_2 flood coming out of the bone would then represent an acid load large enough to turn on the kidney regulation and cause an increased net acid excretion, associated with an increased bicarbonate retention. This could result in a phase of respiratory acidosis lasting from about 20 days to 35 days, which corresponds to the increase in bicarbonate found (Fig. 6). Subsequently, the cycle repeats itself. The second phase of CO_2 storage in the bones, requiring about 20 days, would end at about 55 days and would coincide approximately with the end of the present patrol exposure period. There are probably a number of other factors that may influence the periods of bone CO_2 storage and release and therefore the phase of metabolic and respiratory acidosis. More investigations about the time sequence of these cycles in acid-base balance during prolonged exposure to low levels of CO_2 need to be carried out.

Additional evidence supporting the hypothesis that bone CO_2 storage and release cause cyclic changes in kidney function and acid-base balance is found in observations made during intermittent exposure to CO_2 (Schaefer et al. 1979). Intermittent exposure to increasing CO_2, rising at a constant rate from 0.0 to 3.0% within a period of 15 h, followed by a 9-h period of air breathing for six days, produced a transient filling and emptying of CO_2 stores within a 5-day period, and led to normal alveolar CO_2 levels and gas exchange data on the sixth day. CO_2 accumulated over a 4-day period and was eliminated through an increased urinary H^+ ion excretion, which was associated with an increased urinary calcium excretion on the fourth and fifth days.

The subject in this study was on a liquid diet to obtain balance data on minerals and to ensure that the hydroxyproline excretion would not be influenced by changes in diet. Hydroxyproline excretion remained at the control level throughout the exposure period, indicating that bone resorption based on parathyroid stimulation was not involved in the calcium flood associated with the CO_2 release. The findings of this experiment provide a model for the explanation of the cyclic changes in acid-base balance found during a patrol.

The exposure periods of 60 days on submarines and of 90 days in a laboratory simulation experiment with 1% CO_2 (Messier et al. 1971) were long enough to accommodate cycles of CO_2 uptake and release in the bone. Based on the limited amount of data available on acid-base balance and calcium metabolism during exposure to low levels of CO_2, the approximate time frame for the periods of CO_2 uptake would be 15–25 days, and for the period of CO_2 release, 10–15 days. If only a few blood samples are drawn, which was the case in most of the patrol studies, one might have either a hypocalcemia or hypercalcemia, depending on the period of the cycle during which the samples were taken. This may explain the difference in Gray, Lampert, and Morris's findings of hypocalcemia in the first submarine study (1969), where only a few samples were taken, and cycles of slight hypocalcemia followed by hypercalcemia in the second British submarine study (1973), with exposure to 0.7% CO_2, in which weekly blood samples were obtained (Fig. 10).

There is another biological model, hibernation, in which acidosis and cyclic changes in hyper- and hypocalcemia seem to occur simultaneously. Riedesel (1960), commenting on the contradictatory literature reports of hypocalcemia and hypercalcemia observed during hiber-

nation, suggested that the calcium level may cycle during long-term hibernation and that the data may have been gathered at different points of the cycle.

Confirmatory evidence for the existence of cycles in bone CO_2 uptake and release has recently been obtained from prolonged exposure of guinea pigs to 1% CO_2 (Schaefer et al. 1979). It has been known for some time that the CO_2 store in the bone contains at least two major fractions: 1) carbonate comprising approximately 60–70% of the total CO_2 content and probably located in the lattice of bone crystals; and 2) bicarbonate, accounting for 30% of the total bone CO_2 stores, which seem to be located in the hydration shell of the hydroxyapatitic crystals and appear to be easily exchangeable. Poyart, Freminet, and Bursaux (1975b) determined, in constant infusion experiments using ^{14}C bicarbonate, that approximately 50% of the ^{14}C activity was lost upon heating. Based on their in-vitro studies, Poyart, Bursaux, and Freminet (1975a) concluded that this heat-labile CO_2 fraction may be considered to make up half of the bone bicarbonate pool. We determined both CO_2 fractions, the dry bone CO_2 content (carbonate) and the heat-labile CO_2, which is the difference between fresh bone and dry bone CO_2 content (bicarbonate), in guinea pigs exposed up to 8 weeks to 1% CO_2.

During the first two weeks of exposure, the bicarbonate fraction increased while the carbonate fraction showed a slight decrease. During the third and fourth week, the carbonate fraction rose markedly and bicarbonate fell to control levels; at six and eight weeks carbonate remained at the level attained at four weeks. However, the bicarbonate fraction rose once again. The rapid uptake of CO_2 into the fast exchangeable bone CO_2 fractions (bicarbonate) during the first phase provides support for the Poyart theory (1975a). The hypothesis states that gaseous CO_2 hydrates with bone water to form carbonic acid, which then dissociates into one HCO_3^- and one H^+ ion

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+ \qquad (1)$$

The hydrogen ion is taken up by available carbonate ions in the form of bicarbonate. The decrease in the carbonate fraction, which is associated with a fall in bone calcium and phosphorus during the first week of exposure, indicates the participation of a fraction of the carbonate pool (calcium-phosphate-carbonate complex). During the subsequent period of three and four weeks, reversal takes place; the carbonate fraction increase is associated with a rise in bone calcium and phosphorus. The bicarbonate fraction, on the other hand, declines. Heat-labile CO_2 fell 20 mM/kg, which corresponds to 40 mM/kg of bicarbonate, during this period. If the CO_2 exchange follows the reaction

$$2HCO_3 \rightarrow CO_2 + H_2O + CO_3^{=} \qquad (2)$$

and 20 mM/kg appear in the form of carbonate, the other 20 mM/kg must have been released as gaseous CO_2 into the extracellular space and blood. It is postulated that this internally released CO_2 provided the stimulus for the activation of the renal bicarbonate reabsorption process, resulting in the phase of respiratory acidosis.

Figure 11 presents in a schematic form the time course of cycles in bone buffering, changes in bone CO_2 fractions, and related calcium-phosphorus exchanges, based on animal experiments. The cyclic changes in acid-base balance found in human studies during exposure to

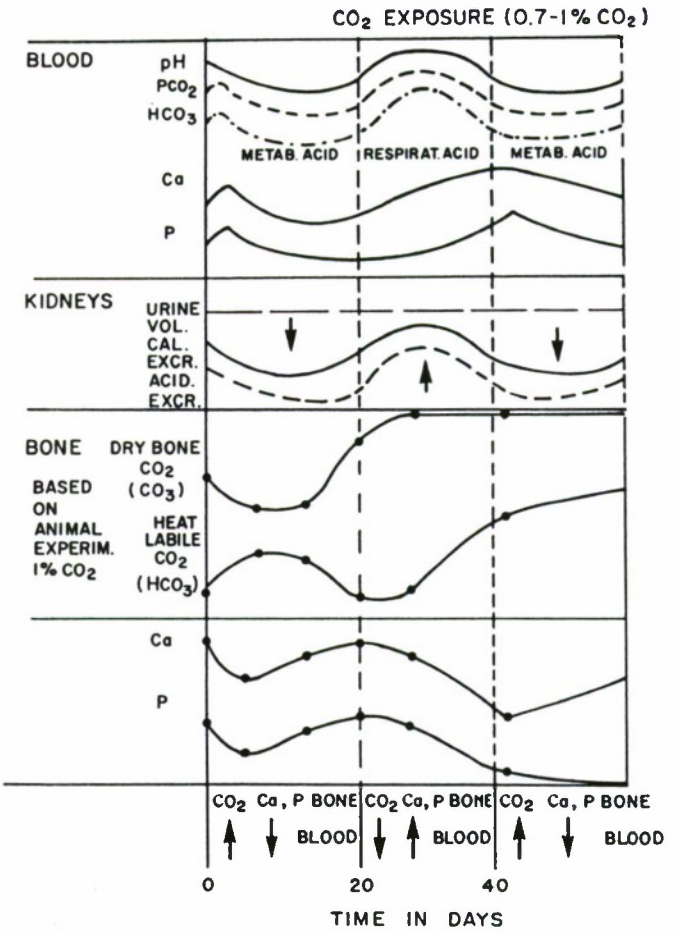

Fig. 11. Cycles in acid-base balance, bone buffering, and renal regulation during prolonged exposure to 0.7%–1% CO_2. Schema is based on data obtained in studies on effects of chronic hypercapnia carried out during patrols and in laboratory experiments. Time course of bone CO_2 and bone calcium and phosphorus is based on animal experiments in which guinea pigs were exposed to 1% CO_2 for 8 weeks (Schaefer et al. 1979b).

0.7%–1.5% CO_2 have been related to cyclic changes in CO_2 and calcium uptake and release by the bones.

The submarine environment contains a number of other factors besides CO_2 that can influence calcium metabolism. Davies and Morris (in this supplement) discuss some of these: the effects of CO_2; reduction in skin synthesis of Vitamin D due to the absence of sunlight; limitations of physical activity; absence of significant trace materials caused by using distilled water; and altered diet habits. They found no evidence that altered dietary habits or distilled water had effects on urinary calcium of the magnitude seen in patrol results. However, a high protein intake in the submariner's diet (120 grams) could cause, according to Johnson, Alcantara, and Linkswiler (1970) and Walker and Linkswiler (1972), an increased urinary calcium

excretion, contrary to the findings obtained in submarine patrols. Moreover, reduced physical activity was found to increase renal calcium excretion in the presence of an ambient CO_2 level of 4% CO_2 (Giannetta and Castleberry 1974). Davies and Morris (1979) obtained unequivocal evidence for the CO_2-induced reduction in urinary calcium excretion in recent chamber studies. After an increased calcium output associated with bed rest on air, they raised the ambient CO_2 level to 0.5% and found a simultaneous reduction in renal calcium excretion. The differences in the results of these two studies are probably related to the difference in the effects of higher and lower CO_2 concentrations on calcium metabolism (summarized by Schaefer (1976)). Exposure to higher CO_2 concentrations results in a continuous hypercalcemia and increased urinary calcium excretion (Schaefer, Hasson, and Niemoeller 1961; Stanmeyer, King, Scofield, and Colby 1962; Heyder 1972).

The significance of the CO_2-induced fall in calcium excretion in the first three weeks of patrol studies is strengthened by findings that reduced physical activity and high protein intake alone cause opposite effects. Davies and Morris (1979) observed a significant decrease in the measured circulating blood levels of 25-hydroxy Vitamin D (25 HCC), indicating a state of hypovitaminosis D, in naval personnel between the beginning and end of a patrol. These authors point out that in their most recent unpublished studies, evidence was obtained showing that fecal excretion of calcium increased, during a 5-week exposure to 0.5% CO_2 and isolation, to a level commensurate with the reduction of 25 HCC levels. The net result of the exposure to the submarine environment with raised ambient CO_2 and artificial light appears therefore to be calcium loss due to increased fecal calcium excretion. These authors concluded that CO_2 contributes to the reduction in urinary calcium excretion during the early part of a patrol and to Vitamin D loss during the later part of a patrol. However, the interaction of CO_2 and Vitamin D hypovitaminosis cannot explain the cycles in calcium excretion reported in this paper; these cycles can only be explained by bone CO_2 uptake and release and associated calcium changes. These cycles in both blood calcium and urinary calcium excretion were not recognized by Davies et al. (1976) and Gray et al. (1973), although they were expressed in the data in their studies.

The theory advanced for the interpretation of the cycles in acid-base balance observed during prolonged exposure to low levels of CO_2 does not fit easily in the framework of accepted concepts of acid-base balance. The major contributions to the understanding of CO_2-induced changes in acid-base balance made by Schwartz and his co-workers (Polak, Haynie, Gordon, Hayes, and Schwartz 1961; Schwartz et al. 1965; van Ypersele, Brasseur, DeConincok 1966) deal only with the effects of high concentrations of CO_2, which cause a rapid and significant renal response. Little attention has been given to the effects of prolonged exposure to low CO_2 concentrations on acid-base balance. In an article on "Concepts of Triple Tolerance to CO_2," (Schaefer 1961a), I have previously pointed to the large differences in the effects of high and low concentrations on the rate of acclimatization. It requires 3–5 days to reach a compensation in pH during exposure to CO_2 concentrations of 3% and above, and much longer times during exposure to lower CO_2 concentrations (Schaefer 1961a). More recent animal studies of prolonged exposure to low CO_2 concentrations have demonstrated that renal reabsorption of bicarbonate, indicated by standard bicarbonate values, becomes less and less effective (Schaefer, Niemoeller, Messier, Heyder, and Spencer 1971). As a matter of fact, during exposure of guinea pigs to 1% CO_2, standard bicarbonate remained below control values for four weeks, indicating a metabolic acidosis (Schaefer et al. 1979).

Figure 12 presents a summary of available data on the rate of acclimatization to chronic hypercapnia based on the time to reach a maximal compensation of pH (arterial or venous blood). The more recent results of Clark, Sinclair, and Welch (1971) and Guillerm and Radzis-

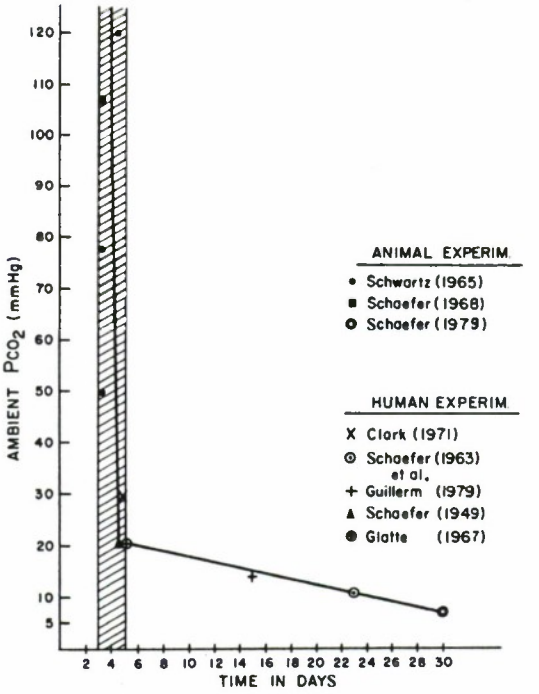

Fig. 12. Time to reach maximal compensation of blood pH during prolonged exposure to different ambient CO_2 tensions. Animal and human experiments.

zewski (1979) are included. There is obviously a systematic difference in the response to levels of CO_2 of 3% and above, compared with that at lower CO_2 concentrations. In the former conditions, the renal regulation (bicarbonte reabsorption) is fully active, while in the latter it becomes less effective and bone buffering, which has a slow time constant, seems to become the dominant factor (Schaefer 1976). This brings up the question of the role of CO_2 stores. In the present concept of gas stores (Farhi 1964), the level of P_{CO_2} in blood or tissue is the determining factor for uptake of CO_2. Guillerm and Radziszewski (in this supplement) have pointed out that on the basis of approximate calculations from data published by Farhi (1964), an overload of a few mmHg of P_{CO_2} is negligible compared with the metabolic CO_2 that is rejected and eliminated. However, it should be pointed out that there are metabolic processes involved in bone CO_2 uptake that clearly are not dependent on P_{CO_2}; the best example is the increase in bone CO_2 that occurs with aging (Poyart et al. 1975a).

Guillerm and Radziszewski (1979) did provide additional data showing opposite effects of higher and lower CO_2 concentrations on blood potassium levels and hematocrits, which are increased during exposure to higher CO_2 concentrations and decreased by lower CO_2 concentrations. This agrees with our observations during exposure to low CO_2 concentrations on patrols (Table 5).

There is a close similarity in the red cell changes observed during exposure to 1.5% CO_2 and those found during exposure to 0.7–1.0% CO_2 on patrols. Under both conditions, red cell sodium increased while red cell potassium decreased. Moreover, red cell calcium increased (Messier et al. 1979). The significance of these changes in relation to a possible inhibition of active transport and red cell permeability needs to be clarified.

Gastric acidity

CO_2 has been shown to be a stimulator of gastric acid secretion in man (Tenney and Naitove 1960; Blakemore and Wolfson 1962). Moreover, gastric secretion was found to increase in dogs during chronic hypercapnia (Phil, Pohl, Dickens, and Glotzer 1967).

Findings of an increased gastric acidity observed during prolonged exposure to 0.80%–0.95% CO_2 are in agreement with the reported effects of acute and chronic hypercapnia on gastric acid secretion and should be taken into consideration in evaluating symptoms of pyrosis. The latter show a rather high incidence during patrols, second only to the incidence of complaints about respiratory symptoms (Table 2).

In view of the established increased incidence of peptic ulcer disease in patients with chronic emphysema and CO_2 retention (Latts, Cummins, and Zieve 1956; Ridgen 1961), it is interesting to note that the incidence of the onset of acute peptic ulcer disease occurring during Polaris submarine patrols has been very low (Wilken 1969).

A summary of changes in respiration, acid-base balance, electrolytes, and calcium metabolism observed during patrols is presented in Table 11.

The results of physiological studies on patrols demonstrate that there is stress on the respiratory system and gastrointestinal system. Incidence of diseases of the respiratory system and gastrointestinal system rank highest in the list of general medical cases in the medical officers' reports (Wilken 1969). Any association of the targets of physiological stress and incidence of disease with the dominant factor in the submarine atmosphere, CO_2, would be strengthened if a decrease in the CO_2 level produced by improved atmosphere control were reflected in a decreased incidence of disease.

This is indeed the case, and these data are presented in Table 12. The incidence of respiratory disease and gastrointestinal disease in the two periods, 1963–1967 and 1967–1973, decreased by 62% and 50%, respectively, simultaneously with a decrease in the level of CO_2 in the submarine atmosphere. Other atmospheric contaminants such as CO and aerosols also

TABLE 12
MEDICAL CASES RESULTING IN SICK DAYS ON PATROL

	1963–1967				1968–1973					
Number of patrols	360				525					
Man-Patrols (140 men/crew)	50,400				73,500					
Average CO_2 concentration during patrols	——				Approximate Decrease −33%					
	Cases		Sick Days		Cases		Sick Days		Cases	Sick Days
1) Respiratory diseases	121	13.9%	480	13.1%	64	7.9%	214	7.8%	−6%	−5.3%
2) Gastrointestinal diseases	139	16.0%	462	12.6%	91	11.2%	237	8.6%	−4.8%	−4.0%

Data for 1963–1967 from Wilken 1969; 1968–1973 data from Tansey et al. 1979.

decreased markedly over this period. During this 10-year period, the CO_2 concentration in the submarine atmosphere decreased approximately 33% (Tansey et al. 1979).

The validity of the observed relationship between decrease in CO_2 concentrations and decrease in incidence of disease can be questioned on the basis of impressions that different standards of medical reporting were used during the 10-year period. On the other hand, no evidence can be marshalled to prove definitely that the observed relationship did not exist. This relationship, between reduction of pollution and decrease in the incidence of sickness, pertains to a healthy population (age range 20–40).

Fallacy of using standard normal clinical values as a reference in long-term exposure studies

Most standard textbook normal values are given for the purpose of differentiating from clearly defined acute pathological conditions. They are not useful for the comparison of subtle changes induced by chronic exposure to different environmental conditions, such as the submarine atmosphere. Moreover, they do not give ranges with respect to circadian cycles. The cyclic changes in acid-base balance, blood pH, bicarbonate, and P_{CO_2} described in this paper fall within the range of normal clinical values. This shows that significant trends, such as those of acid-base balance indicating certain aspects of chronic CO_2 toxicity, are clearly expressed over time (horizontally) within the (vertical) range of so-called normal clinical values.

Outlook for future studies

Evaluation of the medical officers' patrol reports with regard to the prevalence of symptoms pointed to the respiratory system and gastric intestinal system as target organs. Specific physiological studies demonstrated that these two systems were the targets of stress effects produced by prolonged exposure to 0.7–1% CO_2. These stress effects disappeared during the recovery period after the patrols. Whether or not chronic stress effects accumulate over time will be one of the factors determined in the proposed Longitudinal Health Study of Submariners. This should include, in addition to general screening, function studies that are sufficiently sensitive to detect residuals of chronic stress effects.

It is suggested that a special project be instituted in which the three basic elements in submarine medicine: (1) patrol reports; (2) in-patrol studies and pre- and post-patrol studies; and (3) longitudinal health study results are coordinated and the results of the three areas evaluated within the overall framework of preventive aspects of submarine medicine.

These patrol studies, covering a period of 10 years, could not have been carried out without the dedicated assistance and support provided by the staff of the Physiology Branch to the Medical Officers in planning, training, and outfitting the patrols. The analysis of blood and urine samples and data evaluation were also done by the Staff of the Physiology Branch. The following current and former members of the Physiology Branch contributed greatly to the success of this overall program: Charles Carey, James Dougherty, Jr., Arthur A. Messier, Michael Jacey, Elly Heyder, and Carolyn Morgan.

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Schaefer, K. E. 1979. Stress physiologique et hypercapnie chez l'équipage de sous-marins. Undersea Biomed. Res. Sub. Suppl.: S15–S47.—Nous résumons les résultats d'études sur la physiologie des effets hypercapniques observés pendant 13 voyages en sous-marins du type "Polaris". Les concentrations moyennes de CO_2 se situaient entre 0,7 et 1%. CO_2 est l'unique contaminant atmosphérique à bord du sous-marin qui puisse avoir une influence directe sur la respiration dans les

concentrations observées. Nous avons comparé les effets physiologiques produits pendant 42 journées d'exposition à CO_2 (1,5%) au laboratoire à ceux observés pendant les voyages de 50–60 jours. Les effets sur la respiration se ressemblent, comme aussi les effets sur les électrolytes sanguins. La ventilation minute a augmenté de 40–63% à cause de l'augmentation du volume tidale. L'espace mort respiratoire est augmenté de 60%, la capacité vitale légèrement diminuée. Des altérations cycliques de l'équilibre acido-basique ont été observées aussi. Le pH et le taux de bicarbonate sanguin sont diminués pendant les premiers 17 jours d'exposition, sont augmentés pendant les 20 jours suivants, et puis sont diminués de nouveau après 40 jours d'exposition. Ces cycles ne se laissent point expliquer par les connaissances actuelles sur la régulation rénale de l'acidose à CO_2, et ne sont pas observés pendant les expositions expérimentales à 1,5% CO_2. Ces altérations de l'équilibre acido-basique sont peut-être provoquées par des cycles osseux de captation et de libération de CO_2. Les durées des cycles osseux de CO_2 coincident avec celles de l'équilibre acido-basique. La corrélation avec les cycles du métabolisme du calcium est étroite. Des altérations semblables (augmentation du sodium, diminution du potassium) des électrolytes érythrocytaires ont été observées au laboratoire comme chez les sous-mariniers. Le calcium érythrocytaire se trouve aussi augmenté da chez les deux groupes. La signification de ces altérations électrolytiques n'est pas encore bien compris. Une augmentation de l'acidité gastrique s'observe aussi chez les sous-mariniers. Toutes les alterations observées pendant les voyages sont revenues aux valeurs normales pendant les périodes de repos.

respiration
équilibre acido-basique
réponse au CO_2
électrolytes sanguins

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